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FORM PTO-1390 (REV. 5-93) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		CASE NO. 11279/3
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 10/030485
INTERNATIONAL APPLICATION NO. PCT/ES00/00058	INTERNATIONAL FILING DATE February 18, 2000	PRIORITY DATE CLAIMED April 23, 1999

TITLE OF INVENTION
NEURONAL EXOCYTOSIS INHIBITING PEPTIDES AND COSMETIC AND PHARMACEUTICAL COMPOSITIONS CONTAINING SAID PEPTIDES

APPLICANT(S) FOR DO/EO/US
BLANES MIRA et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☒ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)) and/or amendments under Article 34.

Items 11. to 16. Below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☒ A change of power of attorney and/or address letter.
16. ☒ Other items or information: A computer-readable form of the sequence listing in accordance w/ PCT Rule 13.2.

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U.S. APPLICATION NO (If known, see 37 CFR 1.5) 10/030485	INTERNATIONAL APPLICATION NO PCT/ES00/00058	CASE NO 11279/3

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO $890.00		
International preliminary **examination** fee paid to USPTO (37 CFR 1.492(a)(1)) $710 00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international **search** fee paid to USPTO (37 CFR 1.492(a)(2)) $740.00		
Neither international preliminary **examination** fee (37 CFR 1.482) nor international search fee (37 CFR 1.492(a)(3)) paid to USPTO.. ..... $1,040 00		
International preliminary examination **fee** paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT	$890	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	130	

Claims	Number Filed	Number Extra	Rate		
Total Claims	23- 20 =	3	x $ 18.00	$48 00	
Independent Claims	9- 3 =	6	x $ 84.00	$504.00	
Multiple dependent claim(s) if Applicable)		0	+ $280.00		
			TOTAL OF ABOVE CALCUATIONS =	$1572	
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28)				$786.00	
			SUBTOTAL =	$	
Surcharge of $130.00 for furnishing the English translation later than the ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1 492(f)).				$	
			TOTAL NATIONAL FEE=	$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3 28, 3.31), $40.00 per property +					
			TOTAL FEES ENCLOSED=	$	
				Amount to be refunded	$
				charged	$

a. ☒ A check in the amount of $786 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No 23-1925 in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23-1925. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Send All Correspondence to:
Brinks Hofer Gilson & Lione
P.O Box 10395
Chicago, IL 60610

S Mrksich

Signature
K Shannon Mrksich, Ph.D.
Name
36,675
Registration Number

10/030485
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"Express Mail" mailing label number EL746761798 US

Date of Deposit: October 22, 2001

Our Case No. 11279/3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

BLANES MIRA et al.

Serial No. Not Assigned

Filing Date: October 22, 2001

For NEURONAL EXOCYTOSIS INHIBITING PEPTIDES AND COSMETIC AND PHARMACEUTICAL COMPOSITIONS CONTAINING SAID PEPTIDES

Examiner:

Group Art Unit No.

PRELIMINARY AMENDMENT
AND
STATEMENT ACCORDING TO 37 C.F.R. § 1.821(f)

COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please enter the following amendments.

IN THE CLAIMS

Please amend the claims as follows:

2. The peptide according to claim 1, wherein the amino acids of said peptide are D-amino acids or L-amino acids.

3. The peptide according to claim 1, wherein the amino acid at the N-terminus of the peptide is acetylated.

4. The peptide according to claim 1, wherein the amino acid at the C-terminus of the peptide is amidated.

5. The peptide according to claim 1, wherein the peptide further comprises a reversible modification that increases its bioavailability and facilitates its permeation through the blood brain barrier and epithelial tissue.

6. An isolated nucleic acid sequence encoding a peptide having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3.

7. The nucleic acid sequence according to claim 6, wherein said nucleic, acid is selected from the group consisting of bicatenary DNA, monocatenary DNA and RNA.

10. A cell which expresses a peptide having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3.

11. A mixture of peptides comprising:

a) at least a peptide having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3; and

b) at least a peptide having an amino acid sequence consisting of 3 to 30 contiguous amino acids of SEQ. ID. NO. 4.

12. A mixture according to claim 11, comprising:

a) at least a peptide having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3; and

b) at least a peptide having an amino acid sequence selected from the group consisting of amino acid sequence of SEQ. ID. NO. 5 and the amino acid sequence of SEQ. ID. NO. 6.

13. A cosmetic composition comprising

a cosmetically effective amount of a peptide having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3 and

a cosmetically acceptable adjuvant.

14. The cosmetic composition according to claim 13, which further comprises, one or more peptides having an amino acid sequence consisting of 3 to 30 contiguous amino acids of SEQ. ID. NO. 4.

15. A method of treating face wrinkels and/or facial asymmetry comprising

applying a cosmetic composition comprising a cosmetically effective amount of a peptide having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3 and a cosmetically acceptable adjuvant.

16. A pharmaceutical composition comprising

a therapeutically effective amount of a peptide having an amino acid sequence selected from the group consisting of amino acid sequence of SEQ. ID. NO. 2 and the amino acid sequence of SEQ. ID. NO. 3 and

a pharmaceutically acceptable excipient.

17. The pharmaceutical composition according to claim 16, which farther comprises, one or more peptides having an amino acid sequence consisting of 3 to 30 contiguous amino acids of SEQ. ID. NO. 4.

18. The pharmaceutical composition according to claim 16, which further comprises, a drug selected from the group consisting of a neuronal glutamate receptor blocker, a calcium chelating agent, an antioxidant, a free radical scavenger and mixtures thereof.

19. The pharmaceutical composition according to claim 18, which further comprises one or more peptides having an amino acid sequence consisting of 3 to 30 contiguous amino acids of SEQ. ID. NO. 4.

20. A pharmaceutical composition comprising

a therapeutically effective amount of a vector comprising a nucleic acid sequence encoding a peptide having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3 and

an adjuvant or a pharmaceutically acceptable excipient, or a mixture thereof.

21. A method of treating a disease and/or disorder mediated by pathological neuronal exocytosis comprising

administering a pharmaceutical composition comprising a therapeutically effective amount of a peptide having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3 and a pharmaceutically acceptable excipient or an adjuvant or a mixture thereof.

22. A method of treating a disease and/or disorder mediated by pathological neuronal exocytosis, comprising

administering a pharmaceutical composition comprising a therapeutically effective amount of a vector comprising a nucleic acid sequence encoding a peptide having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3, and an adjuvant or a pharmaceutically acceptable excipient, or a mixture thereof.

Please add new claim 23 as follows:

23. The pharmaceutical composition according to claim 18, further comprising one or more neuronal exocytosis inhibitors.

STATEMENT ACCORDING TO 37 C.F.R. § 1.821(f)

Submitted herewith is a sequence listing as part of the above-captioned patent application. Applicants' representative states that the content of the attached paper copy and the attached computer readable copy of the Sequence Listing, submitted in accordance with 37 CFR 1.821(c) and (e), respectively, are the same.

Applicants' representative hereby verifies that the information on the accompanying diskette is identical to the written sequence listing. The enclosed sequence listing does not include any new matter that goes beyond the disclosure in the captioned application as filed.

REMARKS

Claims 1-23 are now pending.

This is an application filed under 35 USC §371. The above amendments were necessary to conform the claims to proper U.S. format. Applicants note that the claims were found novel by the International Preliminary Examining Authority in the concurrently submitted International Preliminary Examination Report dated August 3, 2001. This application is now ready for examination on the merits.

Respectfully submitted,

Date: October 22, 2001

K. Shannon Mrksich, Ph.D.
Reg. No. 36,675
Attorney for Applicants

BRINKS HOFER GILSON & LIONE
P.O. BOX 10395
CHICAGO, IL 60610
(312) 321-4200

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PENDING CLAIMS

1. A peptide having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3.

2. (Amended) The p [P]eptide according to claim 1, wherein the amino acids of said peptide are D-amino acids or L-amino acids.

3. (Amended) The p [P]eptide according to claim 1, wherein the amino acid at the N-terminus of the peptide is acetylated.

4. (Amended) The p [P]eptide according to claim 1, wherein the amino acid at the C-terminus of the peptide is amidated.

5. (Amended) The p [P]eptide according to claim 1, wherein the peptide further [contains] comprises a reversible modification that increases its bioavailability and facilitates its permeation through the blood brain barrier and epithelial tissue.

6. (Amended) An isolated nucleic acid sequence [which codifies] encoding a peptide [according to anyone of claims 1 to 5] having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3.

7. (Amended) The n [N]ucleic acid sequence according to claim 6, wherein said nucleic, acid is selected from the group consisting of bicatenary DNA, monocatenary DNA and RNA.

8. A plasmid which comprises a nucleic acid sequence according to claim 6.

9. An expression vector which comprises a nucleic acid sequence according to claim 6.

10. (Amended) A cell which expresses a peptide [according to anyone of claims 1 to 5] having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3.

11. (Amended) A mixture of peptides comprising:

a) at least a peptide [according to anyone of claims 1 to 5] having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3; and

b) at least a peptide having an amino acid sequence consisting of 3 to 30 contiguous amino acids [contained in] of SEQ. ID. NO. 4.

12. (Amended) A mixture according to claim 11, comprising:

a) at least a peptide [according to anyone of claims 1 to 5] having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3; and

b) at least a peptide having an amino acid sequence selected from the group consisting of amino acid sequence of SEQ. ED. NO. 5 [or] and the amino acid sequence of SEQ. ID. NO. 6.

13. (Amended) A cosmetic composition comprising a cosmetically effective amount of [at least] a peptide [according to anyone of claims 1 to 5, together with at least] having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3 and a cosmetically acceptable adjuvant.

14. (Amended) The c [C]osmetic composition according to claim 13, which further comprises, [optionally,] one or more peptides having an amino acid sequence consisting of 3 to 30 contiguous amino acids [contained in] of SEQ. ID. NO. 4.

15. (Amended) A method of treating [Use of a peptide according to anyone of claims 1 to 5, in the manufacture of a cosmetic composition for the treatment of] face wrinkels and/or facial asymmetry comprising

applying a cosmetic composition comprising a cosmetically effective amount of a peptide having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3 and a cosmetically acceptable adjuvant.

16. (Amended) A pharmaceutical composition comprising

a therapeutically effective amount of[, at least,] a peptide [according to anyone of claims 1 to 5, together with, at least,] having an amino acid sequence selected from the group consisting of amino acid sequence of SEQ. ID. NO. 2 and the amino acid sequence of SEQ. ID. NO. 3 and

a pharmaceutically acceptable excipient.

17. (Amended) The p[P]harmaceutical composition according to claim 16, which farther comprises, [optionally,] one or more peptides having an amino acid sequence consisting of 3 to 30 contiguous amino acids [contained in] of SEQ. ID. NO. 4.

18. The p [P]harmaceutical composition according to claim 16, which further comprises, [optionally,] a drug selected from the group consisting of a neuronal glutamate receptor blocker, a calcium chelating agent, an antioxidant, a free radical scavenger [scavanger] and mixtures thereof [and, optionally, one or more additional neuronal exocytosis inhibitors].

19. (Amended) The p [P]harmaceutical composition according to claim 18, which further comprises[, optionally,] one or more peptides having an amino acid sequence consisting of 3 to 30 contiguous amino acids [contained in] of SEQ. ID. NO. 4.

20. (Amended) A pharmaceutical composition comprising

a therapeutically effective amount of a vector comprising [containing, at least,] a nucleic acid sequence [according to claim 6, coding for a peptide according to anyone of claims 1 to 5, together with, at least,] encoding a peptide having an amino acid sequence

selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3 and

an adjuvant [and/or] or a pharmaceutically acceptable excipient, or a mixture thereof.

21. (Amended) [Use of a peptide according to anyone of claims 1 to 5, in the manufacture of a pharmaceutical composition for the treatment of] A method of treating a disease[s] and/or disorder[s] mediated by pathological neuronal exocytosis comprising

administering a pharmaceutical composition comprising a therapeutically effective amount of a peptide having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3 and a pharmaceutically acceptable excipient or an adjuvant or a mixture thereof.

22. (Amended) [Use of a vector containing, at least, a nucleic acid sequence according to claim 6, .coding for a peptide according to anyone of claims 1 to 5, in the manufacture of a pharmaceutical composition for the treatment of] A method of treating a disease[s]and/or disorder[s] mediated by pathological neuronal exocytosis, comprising

administering a pharmaceutical composition comprising a therapeutically effective amount of a vector comprising a nucleic acid sequence encoding a peptide having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3, and an adjuvant or a pharmaceutically acceptable excipient, or a mixture thereof.

23. (New) The pharmaceutical composition according to claim 18, further comprising one or more neuronal exocytosis inhibitors.

NEURONAL EXOCYTOSIS INHIBITING PEPTIDES AND COSMETIC AND PHARMACEUTICAL COMPOSITIONS CONTAINING SAID PEPTIDES

FIELD OF THE INVENTION

This invention refers to peptides derived from the amino end of protein SNAP-25, useful as inhibitors of neuronal exocytosis, and to their use in cosmetic and/or therapeutic applications, together (optionally) with a peptide derived from the carboxyl end of protein SNAP-25

BACKGROUND OF THE INVENTION

The basis or mechanism for the formation of facial wrinkles is the tensing of the muscles of the epidermis that drag the skin inwards. This muscular tension is the result of hyperactivity of the nerves innervating the facial muscles. Nerve hyperactivity is characterized by the uncontrolled and excessive release of neurotransmitters that excite muscle fibers. Because of this, the molecules that control neuronal exocytosis contribute to relaxing muscular tension, and consequently, to eliminating wrinkles.

Botulinum toxins are a family of bacterial neurotoxins produced by *Clostridium Botulinum* (1) [see section regarding BIBLIOGRAPHY]. 7 different serotypes are known (serotypes A, B, C, D, E, F and G) with an average molecular weight of 150 kDa. These toxins inhibit acetylcholine exocytosis in the neuromuscular junction (nerve-muscle synapse) of the skeletal muscle (1).

At a molecular level, botulinum toxins are proteases that degrade neuronal proteins involved in the exocytosis mechanism activated by the calcium ion (1-3). For example, botulinum toxin A, the one most commonly used clinically and cosmetically [because of its applications in eliminating facial wrinkles and asymmetry, and to mitigate the symptomatology of spastic diseases], cleaves the neuronal protein SNAP-25. This protein (SNAP-25) plays a key role in neurosecretion, as it is involved in the formation of a protein complex (known as SNARE complex or fusion complex), which directs and controls the release of acetylcholine accumulated in vesicles. The nucleus of said fusion complex is made up of proteins SNAP-25 and syntaxin, located in the presynaptic plasma membrane, and protein synaptobrevin (or VAMP), located in the vesicular plasma membrane (4, 5). The main function of the fusion complex is to bring the vesicle loaded with neurotransmitter (acetylcholine) nearer to the presynaptic plasma membrane and put it in contact with same (4, 5). In this way, in response to an

elevated concentration of calcium, the fusion of both plasma membranes is encouraged, thus producing the release of the neurotransmitter. Therefore, said vesicle docking and fusion protein complex (SNARE) is a key target in controlling neurosecretion. Cleaving any of the proteins that make up the fusion complex prevents its assembly, and therefore inhibits vesicle release and neuronal exocytosis.

The power of botulinum toxins and, in particular, serotype A (BOTOX®) to inhibit neurosecretion, as well as their neuronal selectivity (they only act on neurons) is being widely used therapeutically to correct spastic ailments such as dystonias, strabismus, tics, blepharospasm, facial scoliosis, etc. (6 – 13). Botulinum toxin A (botulinum A) is, moreover, an effective agent for eliminating facial wrinkles and asymmetry. In fact, the administration of BOTOX® is the first effective non-surgical therapy to eliminate the signs of aging (6, 7).

Therapeutic and cosmetic treatment with BOTOX® consists of a localized injection of diluted pharmaceutical preparations (botulinum A-hemagglutinin complex, 500 kDa) in the areas where muscular tension is localized. The paralytic effects of the toxin are reversible with an average duration of 6 months (6, 7). The treatment, therefore, requires repeated injections of BOTOX®. The main problem with this treatment is the chance that it may trigger an immune reaction against the pharmaceutical preparation due to the fact that, because of its molecular size, it may be recognized by the patient's immune system. The appearance of antibodies against botulinum A is a serious problem, as it contributes to a clear decrease in the treatment's effectiveness (6-13). This loss of effectiveness in treatment with BOTOX® means the need to increase the preparation's concentration level in later treatments, which in turn produces a potentiation of the immune response. As an alternative, the use of different botulinum toxin (BoTox) serotypes has been discussed, such as BoTox B, BoTox F and BoTox E. Nevertheless, the application of pharmaceutical preparations with different serotypes cannot be considered a solution to the problem, as sooner or later the immune reaction may once again occur. In addition, treatment with botulinum toxins is expensive, mainly because of the lability and instability of the pharmaceutical preparations containing them.

There is, therefore, a pressing need to develop molecules that imitate the paralytic effects of the botulinum toxins, but with much simpler and more stable molecular structures, which do not cause immune reactions, and whose manufacturing cost is economical. Peptide-type molecules comply with these properties.

Amino acid sequences that inhibit neuronal exocytosis have been described. Specifically, it has been proven that peptides with more than 20 amino acids, deriving from the C-terminal sequence of SNAP-25, block the release of catecholamines from permeabilized chromaffin cells (14). Likewise, peptides deriving from the amino acid sequences of proteins syntaxin and VAMP have been described that can also affect the exocytotic process (15). Although these peptides show biological activity, their later development as cosmetic and/or therapeutic agents has not occurred, most likely due to their size, as this complicates their development as useful therapeutic agents and makes it more expensive. Therefore, there is a need to find molecules of a smaller size that can be applied in cosmetics and medicine.

This invention provides a solution to the existing needs which includes the discovery of smaller amino acid sequences, between 3 and 30 amino acids, deriving from the amino end (N-terminal domain) of protein SNAP-25, which inhibit neuronal exocytosis. In addition, it has been discovered that the simultaneous use of peptides deriving from the amino end and from the carboxyl end (C-terminal domain) of SNAP-25 produces a considerable increase in their inhibitory activity, i.e., there is a potentiation of their activity compared to that shown by individual peptides.

Therefore, one object of this invention is a peptide that has a sequence made up of 3 to 30 adjacent amino acids contained on the amino end of protein SNAP-25, which inhibits neuronal exocytosis.

An additional object of this invention is a nucleic acid that essentially codes for one of the peptides provided by this invention. The plasmids and vectors that contain said nucleic acid (also identified as constructions), as well as the cells transformed with said plasmids or vectors that express a peptide of the invention, also constitute additional objects of this invention.

Another additional object of this invention is a mixture of at least one of the peptides provided by this invention and at least one peptide that has a sequence made up of 3 to 30 adjacent amino acids contained on the carboxyl end of protein SNAP-25.

Another additional object of this invention is a cosmetic composition that includes at least one of the peptides provided by this invention. The use of the peptides provided by this invention in the preparation of said cosmetic composition, as well as the method of cosmetic treatment that includes the application of said cosmetic composition, constitute additional objects of this invention.

Another additional object of this invention is a pharmaceutical composition that includes at least one of the peptides provided by this invention, or alternatively, a vector containing a nucleic acid that codes for one of the peptides of the invention. The use of the peptides and vectors (constructions) provided by this invention in the preparation of said pharmaceutical compositions, as well as the method of treating humans or animals encompassed by the application of said cosmetic composition, constitute additional objects of this invention.

Another additional object of this invention is a combination of drugs that includes at least one of the peptides provided by this invention, along with, at least, one drug intended for a second therapeutic target which may be the same as or different from the therapeutic target at which the peptide provided by this invention is aimed.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a peptide deriving from the amino end of protein SNAP-25. More specifically, the invention provides a peptide, henceforth known as the peptide of the invention, which has a sequence of 3 to 30 adjacent amino acids contained in SEQ. ID. No. 1 [see the section regarding the SEQUENCE LIST].

The invention also includes peptides which are substantially homologous to the peptide of the invention. In the sense used in this description, the expression "substantially homologous" means that the peptide in question has a homology level, as far as amino acids are concerned, of at least 60%, and preferably of at least 80%, and even more preferably, of at least 95%.

The invention also includes peptides which are functionally equivalent to the peptide of the invention. In the sense used in this description, the expression "functionally equivalent" means that the peptide in question has at least one of the biological activities of the peptide of the invention, such as, for example, the ability to at least partially inhibit neuronal exocytosis.

In one particular embodiment, the peptide of the invention has a length of 3 to 20 amino acids, and preferably from 6 to 19 amino acids.

The amino acids that make up the structural units of the peptide of the invention may have D- or L-configuration. The amino acid from the amino end may have an acetylated terminal amino group, and the amino acid from the carboxyl end may have an amidated terminal carboxyl group. Therefore, this invention also includes

derivatives of the peptide of the invention in which the amino-terminal end is acetylated and/or in those where the carboxy-terminal end is amidated.

Particular examples of peptides of the invention are those peptides that have sequences of amino acids shown in SEQ. ID No. 2 and SEQ. ID No. 3.

Within the scope of this invention are cosmetically and/or pharmaceutically acceptable salts of the peptide of the invention. The term "cosmetically and/or pharmaceutically acceptable salts" includes salts customarily used to form metal salts or salts formed by adding free acids or bases. The nature of the salt is not critical, as long as it is cosmetically and/or pharmaceutically acceptable. Cosmetically and/or pharmaceutically acceptable salts of the peptide of the invention may be obtained from acids or bases, organic or inorganic, by conventional methods which are well known to technicians in these matters, by making the appropriate acid or base react with the peptide of the invention.

In addition, the peptide of the invention may undergo reversible chemical modifications in order to increase its bioavailability (including stability and fat solubility) and its ease in passing through the blood-brain barrier and epithelial tissue. Examples of such reversible chemical modifications include the esterification of the carboxylate groups of glutamic and aspartic amino acids with an acetyl-methyl group, by which the negative charge of the amino acid is eliminated and its hydrophobicity is increased. This esterification is reversible, as the ester link formed is recognized by intracellular esterases which hydrolyze it, giving back the charge to the aspartic and glutamic residues. The net effect is an accumulation of intracellular peptide, as the internalized, de-esterified peptide cannot cross the cell membrane.

The peptide of the invention can be obtained through conventional methods for solid-phase chemical peptide synthesis, following Fmoc and/or Boc-based methodology (16).

Alternatively, the peptide of the invention can be obtained through conventional methods based on recombinant DNA technology, e.g., through a method that, in brief, includes inserting the nucleic acid sequence that codes for the peptide of the invention into an appropriate plasmid or vector, transforming competent cells for said plasmid or vector, and growing said cells under conditions that allow the expression of the peptide of the invention and, if desired, isolating and (optionally) purifying the peptide of the invention through conventional means known to experts in these matters. The nucleic acid sequence that codes for the peptide of the invention may be easily deduced from

the correspondence that exists between the amino acids and the nucleotide codons that code for such amino acids. In this case, an additional object of the invention is an isolated nucleic acid sequence that codes for the peptide of the invention. In one particular embodiment, said nucleic acid is selected among single-strand DNA, double-stranded DNA, and RNA. Additional objects of this invention are the plasmids and expression vectors that contain said nucleic acid sequence that codes for the peptide of the invention, as well as prokaryotic or eukaryotic cells that express the peptide of the invention. A review of the principles of recombinant DNA technology may be found, for example, in the text book entitled "*Principles of Gene Manipulation: An Introduction to Genetic Engineering,*" R.W. Old & S.B. Primrose, published by Blackwell Scientific Publications, 4th Edition (1989).

The peptide of the invention is able to at least partially inhibit neuronal exocytosis, probably through a mechanism that involves interfering with the assembly of the fusion protein complex (SNARE) and/or its thermal destabilization.

The neuronal-exocytosis (neurosecretion) inhibiting capabilities of the peptides of the invention became evident through a test that evaluates the strength of said peptides in inhibiting the release of catecholamines induced by calcium in chromaffin cells permeabilized with a detergent [see Example 1.2.1]. Briefly, the chromaffin cell cultures are incubated with epinephrine and norepinephrine containing tritium, are permeabilized with digitonin, and stimulated with calcium, and the amount of radioactivity released by the cells to the extracellular medium, which is a reflection of the exocytosis of said tritium-containing catecholamines, is measured.

The hexapeptide of the invention [SEQ. ID. No. 2], at a concentration of 1 mM, blocked approximately 20% of the release of catecholamines (epinephrine and norepinephrine) in permeabilized chromaffin cells, while the peptide with 13 amino acids [SEQ. ID. No. 3], at a concentration of 1 mM, inhibited approximately 35% of the release of catecholamines in the permeabilized chromaffin cells.

The peptides shown in SEQ. ID. No. 5 and SEQ. ID. No. 6, from the carboxyl end of SNAP-25 [SEQ. ID. No. 4], inhibited the secretion induced by Ca^{2+} in chromaffin cells permeabilized with digitonin by approximately 40% when they were used at a concentration of 1mM.

Parallel tests performed using, jointly, at least one peptide from the amino end of SNAP-25, for example, the peptide of SEQ. ID. No. 2 or of SEQ. ID. No. 3, and at least one peptide from the carboxyl end of SNAP-25, for example, the peptide of SEQ. ID.

No. 5 or of SEQ. ID. No. 6, made it evident that the combined use of at least one peptide from the amino end of SNAP-25 and at least one peptide from the carboxyl end of SNAP-25 strengthens the biological activity observed for each of the peptides tested separately.

In one particular case, mixtures of peptides made up of one of the peptides shown in SEQ. ID. No. 2 or in SEQ. ID. No. 3 and one of the peptides shown in SEQ. ID. No. 5 or in SEQ. ID. No. 6, at a concentration of 0.5 mM for each of them, were tested, and an inhibition rate of 55% was obtained in the release of catecholamines in permeabilized chromaffin cells.

Taken all together, these results indicate that both types of peptides, both those from the amino end and those from the carboxyl end, inhibit catecholamine exocytosis, and that the combined use of peptides from the amino end and the carboxyl end strengthens the biological activity observed for each of them separately.

Therefore, the invention also provides a mix of peptides which includes:

(a) at least one peptide of the invention, and

(b) at least one peptide with a sequence of 3 to 30 adjacent amino acids contained in SEQ. ID. No. 4 [henceforth, (COOH) peptide to indicate its relationship with the carboxyl end of SNAP-25].

In one particular embodiment, said mix of peptides is made up of at least one peptide selected from the group formed by the peptides shown in SEQ. ID. No. 2 and in SEQ. ID. No. 3, and at least one peptide selected from the group formed by the peptides shown in SEQ. ID. No. 5 and in SEQ. ID. No. 6.

The ability of the peptides of the invention to interfere with the formation and stability of the fusion complex (SNARE) became evident through the performance of *in vitro* reconstitution tests of the fusion protein complex with recombinant proteins [see Example 1.2.2]. Briefly, protein SNAP-25 was immobilized in 96-well plates, proteins VAMP and syntaxin were added in the presence and/or absence of the peptides of the invention, and the formation of the fusion protein complex (SNARE) was evaluated. The detection of the complex was performed using an antibody against syntaxin (anti-syntaxin), followed by a second, tagged antibody which recognizes the anti-syntaxin antibody. The data obtained seem to indicate that the presence of the peptides of the invention during the assembly of the fusion complex causes a significant decrease in same. Therefore, the mechanism of the neuronal exocytosis inhibiting action seems to

imply that the peptides of the invention interfere with the formation and/or stability of the fusion protein complex (SNARE).

The results obtained with said tests suggest that the peptides of the invention, peptides which are small in size, between 3 and 30 amino acids, deriving from the amino acid sequence from the amino end of SNAP-25, along with (optionally) peptides from the carboxyl end of SNAP-25, act as neuronal exocytosis inhibitors. Given that these peptides imitate the sequences of neuronal proteins involved in exocytosis, their activity is specific, as they only interact with the corresponding neuronal proteins without affecting other cell components.

The action mechanism of the peptides of the invention seems to be similar to that of botulinum toxins, thus affecting the formation and/or stability of the fusion protein complex; so the peptides of the invention can be considered to have cosmetic/therapeutic applications similar to those described for botulinum toxin. Therefore, the peptides of the invention can become effective, stable, safe and economical substitutes for botulinum toxins, both in the treatment of facial wrinkles and/or asymmetry and in the treatment of the symptomatology of spastic diseases, and their use as neuroprotectors in the treatment of neurological disorders and neurodegenerative diseases.

The studies made by the applicants suggest, moreover, the innovative concept of the simultaneous use of peptides from the N-terminal and C-terminal domains of SNAP-25 as neuronal exocytosis modulators.

Taken together, the results obtained with the peptides of the invention, along with their stability and structural simplicity and the chemical diversity that can be obtained, keeping in mind the composition of the amino and carboxyl ends of SNAP-25, give the peptides of the invention great cosmetic and/or therapeutic potential.

The peptides of the invention may be used for pathological neuronal exocytosis-mediated cosmetic and/or therapeutic purposes.

Among the cosmetic applications of the peptides of the invention are the treatment and total or partial elimination of facial wrinkles and/or asymmetry in humans.

The invention provides a cosmetic composition that includes a cosmetically effective amount of at least one peptide of the invention, along with at least one cosmetically acceptable adjuvant. Additionally and optionally, said cosmetic composition may contain one of the peptides identified as (COOH) peptide.

For cosmetic applications, the peptides of the invention may be applied through any medium that produces contact between the peptide and the place where it is to act in a mammal's body, preferably in humans.

The cosmetically effective amount of peptide that should be applied, as well as the dosage for the treatment of facial wrinkles and/or asymmetry with the peptides and/or cosmetic compositions of the invention, will depend on numerous factors, including the age and condition of the person desiring treatment, the severity of the wrinkles and/or facial asymmetry, the method and frequency of application and the particular peptide to be used.

The presentation of the cosmetic compositions containing the peptides of the invention may be in any form that is suitable for application, e.g., solid, liquid or semi-solid, such as creams, ointments, gels or solutions, and the application of these compositions may be by any suitable means, preferably topically, so they will include the cosmetically acceptable adjuvants necessary to make up the desired form of administration. In a preferred and particular embodiment, the peptides of the invention are encapsulated in liposomes, along with (optionally) another or other (COOH) peptide(s), which are added to the other components of the cosmetic preparation. A review of the different cosmetic forms for applying active compounds and of the adjuvants necessary for obtaining same may be found, for example, in the text book "Cosmetología de Harry" (Harry's Cosmetology), Wilkinson & Moore, Ed. Díaz de Santos (1990).

Therefore, an additional object of this invention is the use of the peptides of the invention in the preparation of cosmetic compositions for the treatment of facial wrinkles and/or asymmetry.

The invention also provides a method for the cosmetic treatment of facial wrinkles and/or asymmetry in mammals, preferably humans, which consists of applying a cosmetically effective amount of at least one peptide of the invention to the mammal that has facial wrinkles and/or asymmetry, along with (optionally) one or more (COOH) peptides, preferably in the form of a cosmetic composition containing it.

In addition, the peptides of the invention are suitable for the treatment of spastic diseases, for example, dystonias, strabismus, blepharospasm, facial scoliosis, tics, etc.; and/or as neuroprotectors in the treatment of neurological disorders and/or neurodegenerative diseases.

Among said neurological disorders are acute neurological diseases, for example, those that take place in the first stages of cerebral ischemia. It is a known fact that during an ischemic process an uncontrolled release of the neurotransmitter glutamate takes place in the affected area. This neurotransmitter interacts with specific neuronal membrane receptors causing a massive influx of calcium ions inside the neuron. The intracellular calcium causes the release of more glutamate, thus triggering a chain reaction. Moreover, the massive, prolonged influx of calcium inside the neurons causes their death, which translates into the formation of necrotic tissue in the ischemic zone. Clearly, the progress of the ischemic damage can be stopped, at least partially, if the uncontrolled glutamate exocytosis is controlled. Therefore, the peptides of the invention, because of their ability to inhibit exocytosis, may be suitable for preventing and/or slowing down the neuronal death that is characteristic of an ischemic process, and so would be useful in the treatment of neuropathologies that occur because of excessive glutamate exocytosis, such as, for example, senile dementia, Alzheimer's-related dementia, AIDS-related dementia, epilepsy, amiotrophic sclerosis, multiple/lateral sclerosis, etc. In this case, application in the treatment of neurological diseases would be similar to the one described for botulinum toxin A (18).

The peptides of the invention could therefore form part of a combined therapy (aimed at several therapeutic targets) with the objective of more effectively stopping neurodegeneration.

An additional object of this invention is a pharmaceutical composition which includes a therapeutically effective amount of at least one peptide of the invention, along with at least one pharmaceutically acceptable excipient. In one particular embodiment, said pharmaceutical composition also contains one or more (COOH) peptides. Alternatively, the pharmaceutical composition of the invention may contain a therapeutically effective amount of a vector that contains at least one nucleic acid sequence that codes for a peptide of the invention, along with at least one adjuvant and/or a pharmaceutically acceptable excipient. Said vector may be used in gene therapy.

The active products of the invention (peptides or vectors) may be administered for the treatment of pathological neuronal exocytosis, manifested, for example, by spastic diseases, neurological disorders or neurodegenerative diseases, through any medium that produces contact between the peptide and the place where it is to act in a mammal's body, preferably in humans.

The therapeutically effective amount of the active product of the invention [peptides or vectors (constructions)] that should be administered, as well as the dosage for the treatment of a pathological condition with the peptides and/or pharmaceutical compositions of the invention, will depend on numerous factors, including the age and condition of the patient, the severity of the disturbance or disorder, the method and frequency of administration and the particular peptide to be used.

The presentation of the pharmaceutical compositions that contain the peptides or vectors (constructions) of the invention may be in any form that is suitable for administration, e.g., solid, liquid or semi-solid, such as creams, ointments, gels or solutions, and these compositions may be administered by any suitable means, for example, orally, parenterally or topically, so they will include the pharmaceutically acceptable excipients necessary to make up the desired form of administration. A review of the different pharmaceutical forms for administering medicines and of the excipients necessary for obtaining same may be found, for example, in the "Tratado de Farmacia Galénica" (Treatise on Galenic Pharmacy), C. Faulí i Trillo, 1993, Luzán 5, S.A. Ediciones, Madrid.

As was previously mentioned, the peptides of the invention could form part of a combined therapy for the purpose of more effectively stopping neurodegeneration. In this case, the invention provides a pharmaceutical composition that includes at least one peptide of the invention, along with (optionally) another or other neuronal-exocytosis inhibiting compound(s), and along with at least one drug intended for another therapeutic target, selected from the group formed by a neuronal glutamate receptor blocker, a calcium chelator, an anti-oxidant, a free-radical destroyer and their combinations.

In one particular embodiment, said composition that is useful in combined therapy may contain at least one peptide of the invention, along with (optionally) another or other neuronal exocytosis inhibiting compound(s) and a neuronal glutamate receptor blocker. In another embodiment of this invention, said composition could contain at least one peptide of the invention, along with (optionally) another or other neuronal exocytosis inhibiting compound(s), a neuronal glutamate receptor blocker, a calcium chelator, an anti-oxidant and/or a free-radical destroyer. Among the neuronal exocytosis inhibiting compounds are peptides from the carboxyl end of SNAP-25, identified as (COOH) peptides. Many other examples of compositions may be proposed, all having in common the need to control neurotransmitter exocytosis.

An additional object of this invention is the use of the peptides of the invention or of vectors that contain at least one sequence that codes for a peptide of the invention, in the preparation of a medicine for the treatment of pathological neuronal exocytosis-mediated pathological diseases and/or disorders, such as, for example, spastic diseases, neurological disorders and/or neurodegenerative diseases.

In addition, the invention provides a method for the treatment in mammals of pathological neuronal exocytosis-mediated pathological diseases and disorders such as, for example, spastic diseases, neurological disorders and/or neurodegenerative diseases, which consists of administering to said mammal suffering from said pathological disease or disorder a therapeutically effective amount of at least one peptide of the invention, or of a vector containing at least one DNA sequence that codes for a peptide of the invention, preferably in the form of a pharmaceutical composition that contains it. In one particular embodiment of this invention, said pharmaceutical composition contains, in addition to the peptide or peptides of the invention, one or more (COOH) peptides.

The following examples serve to illustrate the nature of this invention and should not be considered in a restricting sense as regards said invention.

EXAMPLE 1

Neurotransmitter exocytosis inhibiting peptides

1.1 Peptide synthesis

The peptides shown in SEQ. ID. No. 2, SEQ. ID. No. 3, SEQ. ID. No. 5 and SEQ. ID. No. 6 have been synthesized through conventional methods for solid-phase chemical peptide synthesis using Fmoc and/or Boc-based synthetic methodology (16). The resulting peptides were purified by high-performance liquid chromatography (HPLC) and were analyzed by mass spectrometry.

1.2 Evaluation of biological activity

To evaluate the biological activity of the peptides obtained in Example 1.1, a test was developed that evaluates the strength of said peptides in inhibiting the release of catecholamines induced by calcium in chromaffin cells, as well as an *in vitro* reconstitution test of the fusion complex (SNARE).

1.2.1 Inhibition of the release of catecholamines

This test was performed to verify the neuronal exocytosis-inhibiting capabilities of the peptides synthesized in Example 1.1. In this test, the strength of said peptides is evaluated in inhibiting the release of catecholamines (norepinephrine and epinephrine) induced by calcium in chromaffin cells (obtained from suprarenal bovine glands) permeabilized with the detergent digitonin, in accordance with the method described by Gutiérrez *et al.* (1995 and 1997).

Briefly, the chromaffin cell cultures are incubated with [^{3}H] -epinephrine and [^{3}H] -norepinephrine, are permeabilized with 20 μM digitonin, and stimulated with calcium (10 μM), in the presence of the peptides to be tested (separate or mixed), and the amount of radioactivity released by the cells to the extracellular medium, which is a reflection of the exocytosis of [^{3}H] -epinephrine and [^{3}H] -norepinephrine, is measured.

The results obtained in inhibiting the release of catecholamines in permeabilized chromaffin cells were the following:

a) the peptide in SEQ. ID. No. 2, from the amino end of SNAP-25, at a concentration of 1 mM, blocked approximately 20% of the release of catecholamines in permeabilized chromaffin cells;

b) the peptide in SEQ. ID. No. 3, from the amino end of SNAP-25, at a concentration of 1 mM, inhibited approximately 35% of the release of catecholamines in the permeabilized chromaffin cells.

c) the peptides in SEQ. ID. No. 5 and SEQ. ID. No. 6, from the carboxyl end of SNAP-25, at a concentration of 1mM, inhibited the secretion induced by Ca^{2+} in chromaffin cells permeabilized with digitonin by approximately 40%; and

d) mixtures of peptides made up of one of the peptides shown in SEQ. ID. No. 2 or in SEQ. ID. No. 3 and one of the peptides shown in SEQ. ID. No. 5 or in SEQ. ID. No. 6, at a concentration of 0.5 mM for each of them, inhibited the release of catecholamines in permeabilized chromaffin cells by approximately 55%.

Taken together, these results indicate that both types of peptides, both those from the amino end and those from the carboxyl end, inhibit catecholamine exocytosis, and that the combined use of peptides from the amino end and the carboxyl end strengthens the biological activity observed for each of them separately.

1.2.2 *In vitro* reconstitution

This test was performed to determine the ability of the peptides obtained in Example 1.1 to interfere with the formation and stability of the fusion complex (SNARE). The test consists of evaluating the *in vitro* reconstitution of the fusion protein complex with recombinant proteins produced in *Escherichia coli*. The reconstitution tests, based on ELISA (Enzyme-Linked Immuno Assay) methods, involve the immobilization of protein SNAP-25 in 96-well plates and the subsequent formation of the fusion protein complex by adding the proteins VAMP and syntaxin in the presence and/or absence of the peptides of the invention. The detection of the complex was performed using an antibody against protein syntaxin (anti-syntaxin), followed by an antibody which recognizes the anti-syntaxin antibody, covalently tagged with a peroxidase. The amount of fusion protein complex was tracked by adding 1,2-phenylenediamine dichloride, whose reaction with the peroxidase produces a product with an orangish-yellow color that absorbs 492 nm in an acid medium.

The data obtained show that the presence of the peptides obtained in Example 1.1 during the assembly of the fusion complex causes a significant decrease in same. Therefore, the mechanism of the action of said peptides seems to imply that said peptides interfere with the formation and/or stability of the fusion protein complex (SNARE).

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CLAIMS

1. A peptide having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 2 or the amino acid sequence of SEQ. ID. NO. 3.

2. Peptide according to claim 1, wherein the amino acids of said peptide are D-amino acids or L-amino acids.

3. Peptide according to claim 1, wherein the amino acid at the N-terminus of the peptide is acetylated.

4. Peptide according to claim 1, wherein the amino acid at the C-terminus of the peptide is amidated.

5. Peptide according to claim 1, wherein the peptide further contains a reversible modification that increases its bioavailability and facilitates its permeation through the blood brain barrier and epithelial tissue.

6. An isolated nucleic acid sequence which codifies a peptide according to anyone of claims 1 to 5.

7. Nucleic acid sequence according to claim 6, wherein said nucleic acid is selected from bicatenary DNA, monocatenary DNA and RNA.

8. A plasmid which comprises a nucleic acid sequence according to claim 6.

9. An expression vector which comprises a nucleic acid sequence according to claim 6.

10. A cell which expresses a peptide according to anyone of claims 1 to 5.

11. A mixture of peptides comprising:

a) at least a peptide according to anyone of claims 1 to 5; and

b) at least a peptide having an amino acid sequence consisting of 3 to 30 contiguous amino acids contained in SEQ. ID. NO. 4.

12. A mixture according to claim 11, comprising:

a) at least a peptide according to anyone of claims 1 to 5; and

b) at least a peptide having an amino acid sequence selected from the amino acid sequence of SEQ. ID. NO. 5 or the amino acid sequence of SEQ. ID. NO. 6.

13. A cosmetic composition comprising a cosmetically effective amount of at least a peptide according to anyone of claims 1 to 5, together with at least a cosmetically acceptable adjuvant.

14. Cosmetic composition according to claim 13, which further comprises, optionally, one or more peptides having an amino acid sequence consisting of 3 to 30 contiguous amino acids contained in SEQ. ID. NO. 4.

15. Use of a peptide according to anyone of claims 1 to 5, in the manufacture of a cosmetic composition for the treatment of face wrinkels and/or facial asymmetry.

16. A pharmaceutical composition comprising a therapeutically effective amount of, at least, a peptide according to anyone of claims 1 to 5, together with, at least, a pharmaceutically acceptable excipient.

17. Pharmaceutical composition according to claim 16, which further comprises, optionally, one or more peptides having an amino acid sequence consisting of 3 to 30 contiguous amino acids contained in SEQ. ID. NO. 4.

18. Pharmaceutical composition according to claim 16, which further comprises, optionally, a drug selected from the group consisting of a neuronal glutamate receptor blocker, a calcium chelating agent, an antioxidant, a free radical scavanger and

mixtures thereof, and, optionally, one or more additional neuronal exocytosis inhibitors.

19. Pharmaceutical composition according to claim 18, which further comprises, optionally, one or more peptides having an amino acid sequence consisting of 3 to 30 contiguous amino acids contained in SEQ. ID. NO. 4.

20. A pharmaceutical composition comprising a therapeutically effective amount of a vector containing, at least, a nucleic acid sequence according to claim 6, coding for a peptide according to anyone of claims 1 to 5, together with, at least, an adjuvant and/or a pharmaceutically acceptable excipient.

21. Use of a peptide according to anyone of claims 1 to 5, in the manufacture of a pharmaceutical composition for the treatment of diseases and/o disorders mediated by pathological neuronal exocytosis.

22. Use of a vector containing, at least, a nucleic acid sequence according to claim 6, coding for a peptide according to anyone of claims 1 to 5, in the manufacture of a pharmaceutical composition for the treatment of diseases and/o disorders mediated by pathological neuronal exocytosis.

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(71) Solicitante *(para todos los Estados designados salvo US)*: LIPOTEC, S.A. [ES/ES]; Santa Eulalia, 240, Edif. Vanduard, E–08902 Hospitalet de Llobregat (ES).

(72) Inventores; e
(75) Inventores/solicitantes *(sólo US)*: BLANES MIRA, Mª Clara [ES/ES]; Santa Eulalia, 240, Edif. Vanduard, E–08902 Hospitalet de Llobregat (ES). LLOBREGAT HERNANDEZ, Mª Mercedes [ES/ES]; Santa Eulalia, 240, Edif. Vanduard, E–08902 Hospitalet de Llobregat (ES). GIL TEBAR, Ana Isabel [ES/ES]; Santa Eulalia, 240, Edif. Vanduard, E–08902 Hospitalet de Llobregat (ES). FERNANDEZ BALLESTER, Gregorio Joaquín [ES/ES]; Santa Eulalia, 240, Edif. Vanduard, E–08902 Hospitalet de Llobregat (ES). PLANELL CASES, Rosa Mª [ES/ES]; Santa Eulalia, 240, Edif. Vanduard, E–08902 Hospitalet de Llobregat (ES). FERRER MONTIEL, Antonio Vicente [ES/ES]; Santa Eulalia, 240, Edif. Vanduard, E–08902 Hospitalet de Llobregat (ES). VINIEGRA BOVER, Salvador [ES/ES]; Santa Eulalia, 240, Edif. Vanduard, E–08902 Hospitalet de Llobregat (ES). GUTIERREZ PEREZ, Luis Miguel [ES/ES]; Santa Eulalia, 240, Edif. Vanduard, E–08902 Hospitalet de Llobregat (ES). CARBONELL CASTELL, Teresa [ES/ES]; Santa Eulalia, 240, Edif. Vanduard, E–08902 Hospitalet de Llobregat (ES). PEREZ PAYA, Enrique [ES/ES]; Santa Eulalia, 240, Edif. Vanduard, E–08902 Hospitalet de Llobregat (ES).

(74) Mandatario: FERNANDEZ PRIETO, Angel; Columela, 5–5º, E–28001 Madrid (ES).

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(54) Título: PEPTITOS INHIBIDORES DE LA EXOCITOSIS NEURONAL, COMPOSICIONES COSMETICAS Y FARMACEUTICAS QUE LOS CONTIENEN

(57) Abstract

The peptide has a sequence of 3 to 30 adjacent aminoacids from the amino end of protein SNAP–25 and is useful as neuronal exocytosis inhibitor. The cosmetic and pharmaceutical compositions contain said peptide and optionally one or more peptides from the carboxyl end of SNAP–25. Said compositions are suitable for the treatment of facial wrinkles and asymmetry and pathological neuronal exocytosis–mediated pathological disorders and alterations.

(57) Resumen

El péptido tiene una secuencia de 3 a 30 aminoácidos contiguos procedentes del extremo amino de la proteína SNAP–25 y es útil como inhibidor de la exocitosis neuronal. Las composiciones cosméticas y farmacéuticas comprende dicho pépdido, opcionalmente junto con uno o más péptidos procedentes del extremo carboxilo de la SNAP–25. Las composiciones son adecuadas para el tratamiento de las arrugas faciales, de la asimetría facial y de trastornos y alteraciones patológicas mediadas por una exocitosis neuronal patológica.

Case No.

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled NEURONAL EXOCYTOSIS INHIBITING PEPTIDES AND COSMETIC AND PHARMACEUTICAL COMPOSITIONS CONTAINING SAID PEPTIDES, the specification of which:

☒ is attached hereto.

☐ was filed on _____ as Application Serial No. _____.

☐ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
PCT/ES00/00058	PCT	February 18, 2000	☒	☐
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
ES1999 00000844	Spain	April 23, 1999	☒	☐
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status-patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

100

Inventor's Signature		Date: 10/10/01
Full name of sole or first inventor	Mª Clara Blanes Mira	
Residence	Spain	
Citizenship	Spanish	
Post Office Address	Santa Eulalia, 240, Edif. Vanduard, E-08902, Hospitalet de Llobregat, Spain	

ESX

Case No.

200 Inventor's Signature — Date: 17/10/2001
Full name of second joint inventor, if any: Mª Mercedes Llobregat Hernandez
Residence: Spain
Citizenship: Spanish
Post Office Address: Santa Eulalia, 240, Edif. Vanduard, E-08902, Hospitalet de Llobregat, Spain ESY

300 Inventor's Signature — Date: 15/10/2001
Full name of third joint inventor: Ana Isabel Gil Tebar
Residence: Spain
Citizenship: Spanish
Post Office Address: Santa Eulalia, 240, Edif. Vanduard, E-08902, Hospitalet de Llobregat, Spain

400 Inventor's Signature — Date: 17/10/2001 ESY
Full name of sixth joint inventor: Gregorio Joaquin Fernandez Ballester
Residence: Spain
Citizenship: Spanish
Post Office Address: Santa Eulalia, 240, Edif. Vanduard, E-08902, Hospitalet de Llobregat, Spain ESY

500 Inventor's Signature — Date: 10-10-2001
Full name of fourth joint inventor: Rosa Mª Planell Cases
Residence: Spain
Citizenship: Spanish
Post Office Address: Santa Eulalia, 240, Edif. Vanduard, E-08902, Hospitalet de Llobregat, Spain ESY

600 Inventor's Signature — Date: 10-10-2001
Full name of fifth joint inventor: Antonio Vicente Ferrer Montiel
Residence: Spain
Citizenship: Spanish
Post Office Address: Santa Eulalia, 240, Edif. Vanduard, E-08902, Hospitalet de Llobregat, Spain ESY

700 Inventor's Signature — Date: 15-10-2001
Full name of sixth joint inventor: Salvador Viniegra Bover
Residence: Spain
Citizenship: Spanish ESY
Post Office Address: Santa Eulalia, 240, Edif. Vanduard, E-08902, Hospitalet de Llobregat, Spain

800 Inventor's Signature — Date: 19-10-2001
Full name of seventh joint inventor: Luis Miguel Gutierrez Perez
Residence: Spain
Citizenship: Spanish ESY
Post Office Address: Santa Eulalia, 240, Edif. Vanduard, E-08902, Hospitalet de Llobregat, Spain

900 Inventor's Signature — Date: 18/10/2001
Full name of sixth joint inventor: Teresa Carboncll Castelló
Residence: Spain
Citizenship: Spanish
Post Office Address: Santa Eulalia, 240, Edif. Vanduard, E-08902, Hospitalet de Llobregat, Spain
ESY

1800

Inventor's Signature — E. Pérez Paya — Date: 18/10/2001

Full name of seventh joint inventor — Enrique Perez Paya

Residence — Spain

Citizenship — Spanish

Post Office Address — Santa Eulalia, 240, Edif. Vanduard, E-08902, Hospitalet de Llobregat, Spain ESX

BRINKS HOFER GILSON & LIONE
P.O. Box 10395
Chicago, IL 60610
(312) 321-4200

Case No. _____

Inventor(s): Mª Clara Blanes Mira et al.

Title: NEURONAL EXOCYTOSIS INHIBITING PEPTIDES AND COSMETIC AND PHARMACEUTICAL COMPOSITIONS CONTAINING SAID PEPTIDES

POWER OF ATTORNEY

The specification of the above-identified patent application:

☒ is attached hereto
☐ was filed on _____ as application Serial No. _____

I hereby revoke all previously granted powers of attorney in the above-identified patent application and appoint the following attorneys to prosecute said patent application and to transact all business in the Patent and Trademark Office connected therewith:

K. Shannon Mrksich 36,675
Justin B. Rand 48,552
Greg M. Zinkl 48,492

Please address all correspondence and telephone calls to K. Shannon Mrksich, Ph.D. in care of:

Brinks Hofer Gilson & Lione
P.O. Box 10395
Chicago, IL 60610
(312)321-4200

The undersigned hereby authorizes the U.S. attorneys named herein to accept and follow instructions from _____ as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorneys named herein will be so notified by the undersigned.

LIPOTEC, S.A., a corporation, certifies that it is the assignee of the entire right, title and interest in the patent application identified above by virtue of either:

☒ An assignment from the inventor(s) of the patent application identified above, a copy of which is attached hereto.
OR

☐ An assignment from the inventor(s) of the patent application identified above. The assignment was recorded in the Patent and Trademark Office at Reel _____, frame _____.
OR

☐ A chain of title from the inventor(s), of the patent application identified above, to the current assignee as shown below:

1. From _____ To: _____
The document was recorded in the Patent and Trademark Office at
Reel _____, frame _____, or a copy thereof is attached.

2. From _____ To: _____
The document was recorded in the Patent and Trademark Office at
Reel _____, frame _____, or a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet.

The undersigned has reviewed the assignment or all the documents in the chain of title of the patent application identified above and, to the best of undersigned's knowledge and belief, title is in the assignee identified above.

The undersigned (whose title is supplied below) is empowered to act on behalf of the assignee.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature _____ Date: 15-10-2001
Name: ANTONIO PARENTE DUEÑA
Title: GENERAL MANAGER

Lipotec S.A.
PEPTIDES & DELIVERY SYSTEMS
P.P.

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: UNIVERSIDAD MIGUEL HERNANDEZ

(B) STREET: Monóvar s/n

(C) CITY: Elche

(D) PROVINCE: Alicante

(E) COUNTRY: ES

(F) POSTAL (ZIP) CODE: 03206

(G) TELEPHONE NO.: 96-665-8727

(H) FAX NO.: 96-665-8680

(ii) TITLE OF THE INVENTION:

NEURONAL EXOCYTOSIS INHIBITING PEPTIDES AND COSMETIC AND PHARMACEUTICAL COMPOSITIONS CONTAINING SAID PEPTIDES

(iii) NUMBER OF SEQUENCES: 6

(iv) COMPUTER-READABLE FORMAT:

(A) TYPE OF MEDIUM: Floppy disk

(B) COMPUTER: IBM-compatible PC

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (OEP)

(2) INFORMATION ABOUT SEQ. ID. NO. 1

(i) CHARACTERISTICS OF THE SEQUENCE:

(A) LENGTH: 82 amino acids

(B) TYPE: amino acid

(C) NUMBER OF CHAINS: single

(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: Peptide

(xi) DESCRIPTION OF THE SEQUENCE: SEQ. ID. NO. 1

Ala Glu Asp Ala Asp Met Arg Asn Glu Leu Glu Glu Met Gln Arg Arg
1 5 10 15

Ala Asp Gln Leu Ala Asp Glu Ser Leu Glu Ser Thr Arg Arg Met Leu
20 25 30

Gln Leu Val Glu Glu Ser Lys Asp Ala Ile Arg Thr Leu Val Met Leu
35 40 45

Asp Glu Gln Gly Glu Gln Leu Glu Arg Ile Glu Glu Gly Met Asp Gln
50 55 60

Ile Asn Lys Asp Met Lys Glu Ala Glu Lys Asn Leu Thr Asp Leu Gly
65 70 75 80

Lys Phe

(2) INFORMATION ABOUT SEQ. ID. NO. 2

(i) CHARACTERISTICS OF THE SEQUENCE:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) NUMBER OF CHAINS: single

(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: Peptide

(xi) DESCRIPTION OF THE SEQUENCE: SEQ. ID. NO. 2

Glu Glu Met Gln Arg Arg
1 5

(2) INFORMATION ABOUT SEQ. ID. NO. 3

(i) CHARACTERISTICS OF THE SEQUENCE:

(A) LENGTH: 13 amino acids

(B) TYPE: amino acid

(C) NUMBER OF CHAINS: single

(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: Peptide

(xi) DESCRIPTION OF THE SEQUENCE: SEQ. ID. NO. 3

Glu Leu Glu Glu Met Gln Arg Arg Ala Asp Gln Leu Ala
1 5 10

(2) INFORMATION ABOUT SEQ. ID. NO. 4

(i) CHARACTERISTICS OF THE SEQUENCE:

(A) LENGTH: 86 amino acids

(B) TYPE: amino acid

(C) NUMBER OF CHAINS: single

(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: Peptide

(xi) DESCRIPTION OF THE SEQUENCE: SEQ. ID. NO. 4

Val Asp Glu Arg Glu Gln Met Ala Ile Ser Gly Gly Phe Ile Arg Arg
1 5 10 15

Val Thr Asn Ala Arg Glu Asn Glu Glu Met Asp Glu Asn Leu Glu Gln
20 25 30

Val Ser Gly Ile Leu Gly Asn Leu Arg His Met Ala Leu Asp Met Gly
35 40 45

Asn Glu Ile Asp Thr Gln Asn Arg Gln Ile Asp Arg Ile Met Glu Lys
50 55 60

Ala Asp Ser Asn Lys Thr Arg Ile Asp Glu Ala Asn Gln Arg Ala Thr
65 70 75 80

Lys Met Leu Gly Ser Gly
85

(2) INFORMATION ABOUT SEQ. ID. NO. 5

(i) CHARACTERISTICS OF THE SEQUENCE:

(A) LENGTH: 18 amino acids

(B) TYPE: amino acid

(C) NUMBER OF CHAINS: single

(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: Peptide

(xi) DESCRIPTION OF THE SEQUENCE: SEQ. ID. NO. 5

Arg Ile Met Glu Lys Ala Asp Ser Asn Lys Thr Arg Ile Asp Glu Ala
1 5 10 15

Asn Gln

(2) INFORMATION ABOUT SEQ. ID. NO. 6

(i) CHARACTERISTICS OF THE SEQUENCE:

(A) LENGTH: 19 amino acids

(B) TYPE: amino acid

(C) NUMBER OF CHAINS: single

(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: Peptide

(xi) DESCRIPTION OF THE SEQUENCE: SEQ. ID. NO. 6

Ala Asp Ser Asn Lys Thr Arg Ile Asp Glu Ala Asn Gln Arg Ala Thr
1 5 10 15

Lys Met Le